

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions and listings of claims in the application:

LISTING OF CLAIMS:

1. (currently amended): A method for classifying and counting leukocytes, which comprises:
 - (1) a step of staining cells in a sample obtained from a hematological sample by treatment with a hemolytic agent, with a fluorescent dye which can make a difference in the fluorescence intensity at least among mature leukocytes, leukocytes with abnormal DNA amount and immature leukocytes;
 - (2) a step of introducing the sample containing the stained cells into a flow cytometer to measure first scattered light, second scattered light different from the first scattered light and fluorescence of the respective cells;
 - (3) a step of ~~classifying a first group and a second group utilizing a difference in the intensity of a obtaining scattered light peak intensities and a difference in the scattered light width, the first group including leukocytes and second group including coincidence cells and platelet clumps widths of the respective cells based on the measured first scattered light, obtaining scattered light intensities of the respective cells based on the measured second scattered light, and obtaining fluorescence intensities of the respective cells based on the measured fluorescence light;~~
 - (4) ~~a step of classifying the cells into a first group and a second group based on the scattered light peak intensities and the scattered light widths, the first group including leukocytes and the second group including coincidence cells and platelet clumps;~~

(5) a step of classifying and counting the leukocytes included in the first group into mature leukocytes, leukocytes with abnormal DNA amount and immature leukocytes, utilizing a difference in based on the scattered light intensity intensities and a difference in the fluorescence intensity intensities of the leukocytes classified in the step (3) included in the first group; and

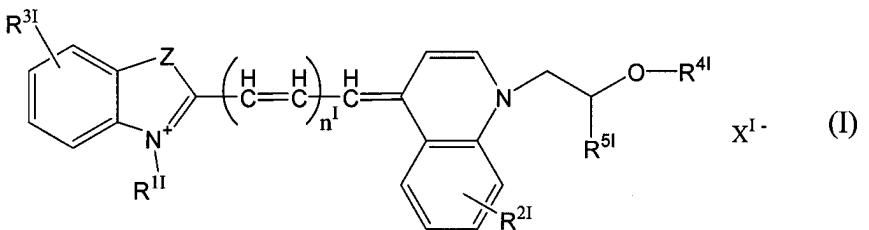
(6) a step of counting the classified mature leukocytes, the classified leukocytes with abnormal DNA amount and the classified immature leukocytes.

2. (original): The method according to claim 1, which further comprises a step of calculating a ratio of mature leukocytes or immature leukocytes relative to leukocytes with abnormal DNA amount from a number of leukocytes with abnormal DNA amount and a number of mature leukocytes or immature leukocytes.

3. (previously presented): The method according to claim 1, which further comprises a step of calculating a ratio of immature leukocytes relative to mature leukocytes from a number of mature leukocytes and a number of immature leukocytes.

4 - 5. (canceled).

6. (previously presented): The method according to ~~any one of claims 1 to 5~~ claim 1, wherein the fluorescent dye is selected from the group consisting of a compound represented by the formula (I):

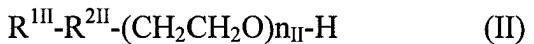


(wherein R¹¹ is a hydrogen atom or a lower alkyl group; R²¹ and R³¹ each is a hydrogen atom, a lower alkyl group or a lower alkoxy group; R⁴¹ is a hydrogen atom, an acyl group or a lower alkyl group; R⁵¹ is a hydrogen atom or a lower alkyl group which may be substituted; Z is sulfur atom, oxygen atom, or carbon atom which is substituted by a lower alkyl group; n¹ is 1 or 2; and X¹⁻ is an anion), ethidium bromide, propidium iodide, ethidium-acridine heterodimer, ethidium azide, ethidium homodimer-1, ethidium homodimer-2, ethidium monoazide, TOTO-1, TO-PRO-1, TOTO-3, and TO-PRO-3.

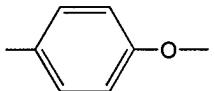
7. (previously presented): The method according to claim 1, wherein the hemolytic agent comprises the following components:

- (1) a polyoxyethylene nonionic surfactant;
- (2) a solubilizing agent to give damage to cell membrane of blood corpuscles and reduce their size;
- (3) an amino acid; and
- (4) a buffer by which pH and osmotic pressure of the liquid are adjusted to 5.0 - 9.0 and 150-600 mOsm/kg, respectively.

8. (previously presented): The method according to claim 7, wherein the polyoxyethylene nonionic surfactant comprises a compound represented by the following formula (II):



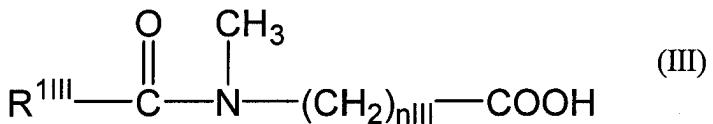
(wherein R^{III} represents a C₉₋₂₅ alkyl, alkenyl or alkynyl group; R^{2II} represents -O-,



or -COO-; and n_{II} is 10-40).

9. (previously presented): The method according to claim 7, wherein the solubilizing agent is a compound selected from the group consisting of

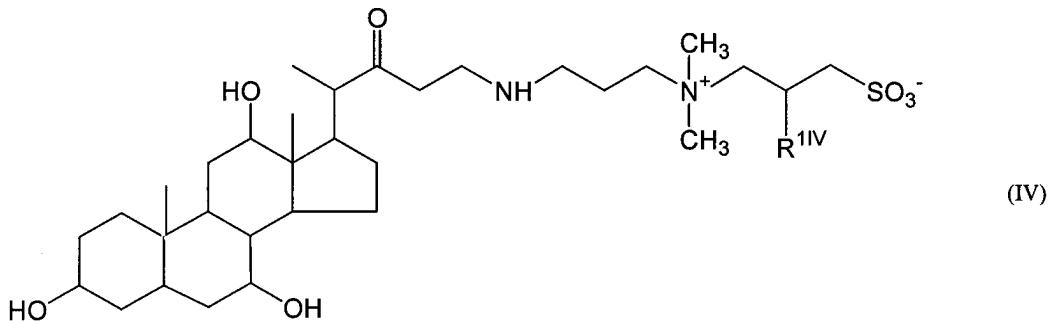
a sarcosine derivative of the formula (III):



(wherein R^{III} is a C₁₀₋₂₂ alkyl group; and n^{III} is 1-5)

or salts thereof;

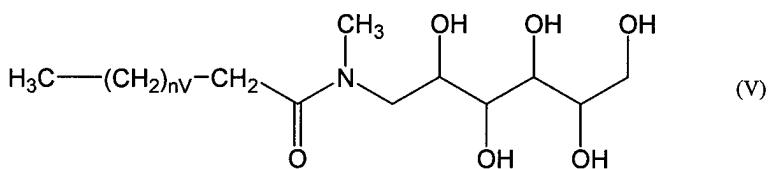
a cholic acid derivative of the formula (IV):



(wherein R^{1IV} is a hydrogen atom or a hydroxy group);

and

a methylglucanamide of the formula (V):



(wherein n^V is 5-7).

10. (previously presented): The method according to claim 1, wherein scattered light to be measured is selected from forward low angle scattered light, forward high angle scattered light and side scattered light.

11. (withdrawn): A system for classifying and counting leukocytes which comprises:
a flow cytometer comprising an orifice portion in which a sample for measurement
passes through, a light source which irradiates light to the orifice portion, a first light receiving
portion which receives scattered light emitted from the orifice portion and a second light
receiving portion which receives fluorescence emitted from the orifice portion, said sample for
measurement being prepared by a step of mixing a hematological sample with a hemolytic agent
and a step of staining cells in the resulting mixture with a fluorescent dye which can make a
difference in the fluorescence intensity at least among mature leukocytes, leukocytes with
abnormal DNA amount and immature leukocytes; and
an analyzing part in which the sample for measurement is analyzed by a step of
classifying a first group and a second group utilizing a difference in the intensity of a scattered
light peak and a difference in the scattered light width, the first group including leukocytes and
the second group including coincidence cells and platelet clumps and a step of classifying and
counting mature leukocytes, leukocytes with abnormal DNA amount and immature leukocytes,
utilizing a difference in the intensity and a difference in the fluorescence intensity of leukocytes
classified.

12. (new): The method according to claim 1, wherein the classifying step (5) is
performed so as to classify the classified mature leukocytes into at least three groups based on
the scattered light intensities.

13. (new): The method according to claim 1, wherein the classifying step (5) is performed so as to classify the classified immature leukocytes into at least two groups based on the scattered light intensities.